

AMENDMENTS TO THE CLAIMS:

Please amend the claims as follows:

1. (Currently Amended) A glutamine-auxotrophic human cell transfected with

(a) ~~[[an]]~~a first exogenous DNA sequence comprising a DNA sequence encoding
~~a sialylated protein or an exogenous DNA sequence capable of altering the expression~~
~~of an endogenous gene encoding a sialylated protein, and which exogenous and a DNA~~
~~sequence encoding further comprises~~ a selectable marker selected from the group
consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate
transcarbamylase, metallotionein-1, ornithine decarboxylase, P-glycoprotein,
ribonucleotide reductase, thymidine kinase and xanthine-guanine phosphoribosyl
transferase, and

(b) ~~[[an]]~~a second exogenous DNA sequence encoding a glutamine synthetase
as a selectable marker,

wherein ~~[[these]]~~said first exogenous DNA sequence and said second
exogenous DNA sequence~~[[s]]~~ are located on different ~~more than one~~ DNA constructs,
~~wherein and said DNA constructs being~~ being ~~[[is a]]~~ vectors, and

wherein the rate of synthesis of said sialylated protein is elevated when said
transfected cell is grown in a glutamine-free media as compared to a control cell not
containing said second exogenous DNA sequence grown in media containing
glutamine, and

wherein said transfected cell produces a reduced concentration of ammonia in a
glutamine-free media as compared to the concentration of ammonia said control cell

produces in media containing glutamine ~~is capable of producing said protein and is capable of growing in a glutamine-free and serum-free medium.~~

Claim 2. (Cancelled)

3. (Previously Presented) The glutamine-auxotrophic human cell of claim 1, wherein the glutamine-auxotrophic human cell is an immortalized glutamine-auxotrophic human cell.

4. (Original) The glutamine-auxotrophic human cell of claim 3, wherein the immortalized glutamine-auxotrophic human cell is a human fibrosarcoma cell.

5. (Original) The glutamine-auxotrophic human cell of claim 4, wherein the human fibrosarcoma cell is a HT1080 cell line.

6. (Previously Presented) The glutamine-auxotrophic human cell of claim 1, wherein the transfected cell is anchorage-independent and capable of growing in suspension in serum-free and glutamine-free medium.

7. (Previously Presented) A process for producing a sialylated protein comprising the steps of

a) culturing a glutamine-auxotrophic human cell according to claim 1 in a serum-free culture medium under conditions suitable for expression of said protein and

b) recovering said protein.

Claim 8. (Canceled)

Claim 9. (Canceled)

10. (Currently Amended) The process of claim 7 wherein the culture medium is ~~serum-free and/or~~ glutamine free.

Claim 11. (Canceled)

Claim 12. (Canceled)

Claim 13. (Canceled)

14. (Currently Amended) The process of claim ~~[[11]]~~10 wherein sialylation is defined by N-glycan charge.

15. (Previously Presented) The process of claim 14 wherein said sialylated protein comprises tri, tetra- or pentasialo glycoforms of said N-glycan.

Claim 16. (Canceled)

17. (Currently Amended) The glutamine-auxotrophic human cell of claim 1 wherein sialylation is defined by N-glycan charge.

18. (Currently Amended) The glutamine-auxotrophic human cell of claim 17 wherein said sialylated protein comprises tri, tetra- or pentasialo glycoforms of said N-glycan.

19. (Previously Presented) The process of claim 7, wherein the glutamine-auxotrophic human cell is an immortalized glutamine-auxotrophic human cell.

20. (Previously Presented) The process of claim 19, wherein the immortalized glutamine-auxotrophic human cell is a human fibrosarcoma cell.

21. (Currently Amended) The glutamine-auxotrophic human cell of claim 1 wherein the sialylated protein is Erythropoietin.

22. (Currently Amended) The glutamine-auxotrophic human cell of claim 21 wherein the Erythropoietin is human Erythropoietin.

23. (Currently Amended) The process according to claim ~~[[11]]~~10 wherein the sialylated protein is Erythropoietin.

24. (Previously Presented) The process according to claim 23 wherein the Erythropoietin is human Erythropoietin.

Claims 25-26. (Canceled)

27. (Previously Presented) The process of claim 20, wherein the human fibrosarcoma cell is a HT1080 cell line.

28. (Currently Amended) A method ~~of producing to produce the~~ glutamine-auxotrophic human cell of claim 1 or 17 comprising ~~the steps of~~

~~(a) firstly-transfecting [[said]]a glutamine-auxotrophic human cell with said different DNA constructs to produce a transfected cell exogenous DNA sequence encoding a protein or~~

~~an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein, and which exogenous DNA sequence further comprises a selectable marker selected from the group consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidin kinase and xanthin-guanine phosphoribosyl~~

~~and~~

~~(b) secondly, transfecting the cell obtained in step (a) with an exogenous DNA sequence encoding a glutamine synthetase, and~~

~~[[[c)]] culturing the transfected cell obtained in step (b) in glutamine-free medium,~~

such that the rate of synthesis of the sialylated protein is elevated as compared to a control cell not containing said second exogenous DNA sequence grown in media containing glutamine, and

such that the transfected cell produces a reduced concentration of ammonia in the glutamine-free media as compared to the concentration of ammonia the control cell produces in media containing glutamine.

29. (Currently Amended) The method of claim 28, wherein said transfected cell is further adapted to the growth in serum free medium.

Claims 30-31. (Cancelled)

32. (new) A method of increasing sialylation and/or N-glycan charge of a glycosylated protein expressed by a glutamine auxotrophic human cell which comprises transfecting a glutamine auxotrophic human cell with an exogenous DNA sequence encoding a glutamine synthetase to produce a transfected glutamine auxotrophic human cell and culturing said transfected glutamine auxotrophic human cell in a glutamine-free media such that said sialylation and/or N-glycan charge of said glycosylated protein is increased.

33. (new) The method of claim 32 wherein said glycosylated protein is encoded by an exogenous DNA sequence and is recovered from the culture of said transfected glutamine auxotrophic human cell.

34. (new) The method according to claim 32, wherein said glutamine auxotrophic human cell or said transfected glutamine auxotrophic human cell is further transfected with an amplifiable gene encoding an enzyme, wherein said enzyme is dihydrofolate

reductase (DHFR), adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase or xanthine-guanine phosphoribosyl transferase.

35. (new) The method according to claim 33, wherein said glutamine auxotrophic human cell or said transfected glutamine auxotrophic human cell is further transfected with an amplifiable gene encoding an enzyme, wherein said enzyme is dihydrofolate reductase (DHFR), adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase or xanthine-guanine phosphoribosyl transferase.